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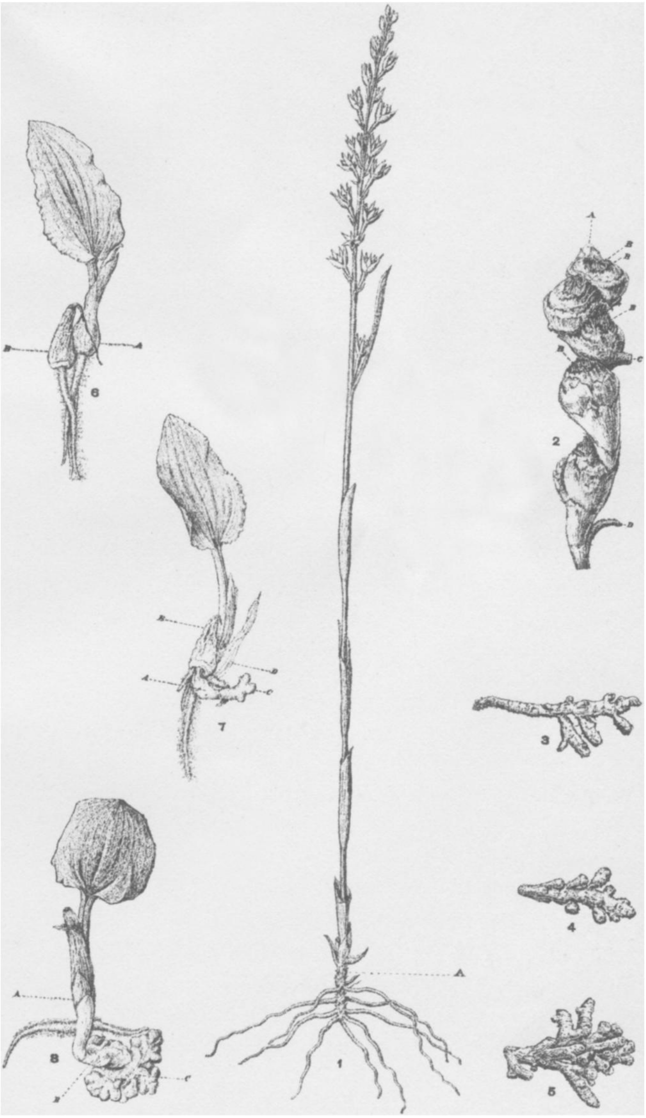

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MACDOUGAL ON SYMBIOSIS.

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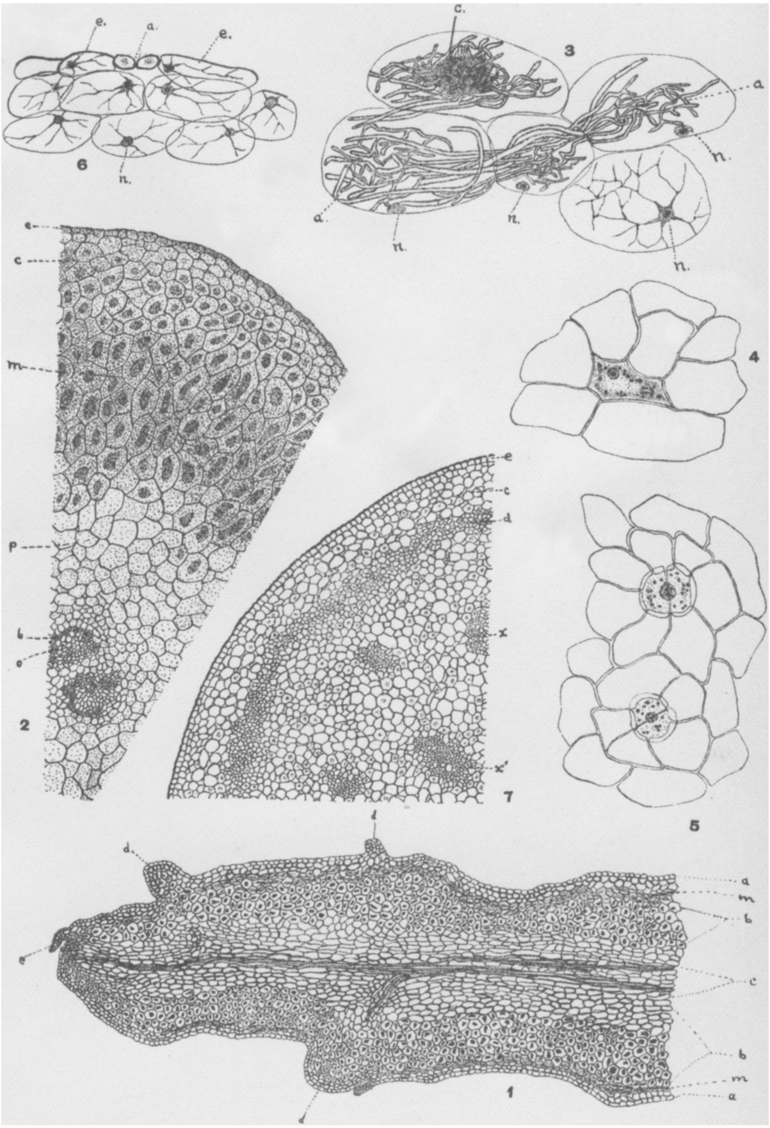

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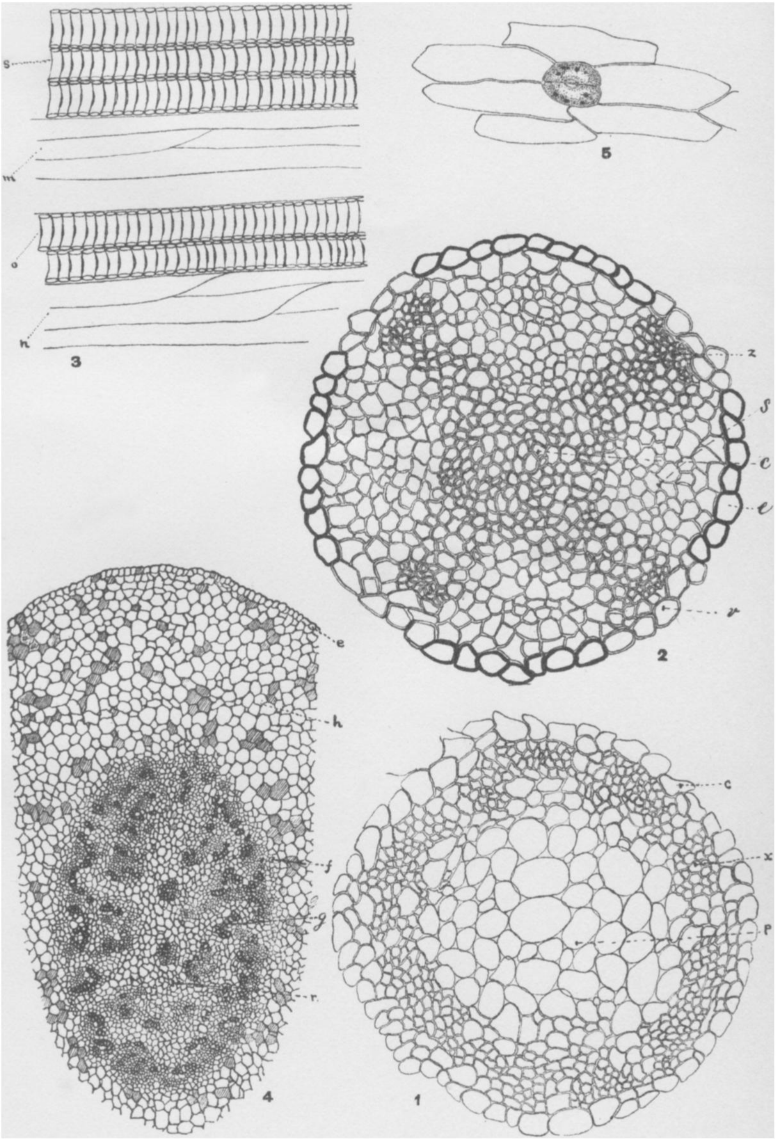

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Symbiosis and Saprophytism

BY DANIEL TREMBLY MACDOUGAL

[PLATES 367–369]

GENERAL DISCUSSION

It is customary to designate all chlorophylless seed-forming species which have no nutritive connection with other vascular plants as saprophytes, or more exactly, holosaprophytes (allotropic or heterotropic forms according to Pfeffer's classification), and others of similar physiological tendencies as hemisaprophytes (mixotropic forms), without regard to the nutritive unions formed by the roots or absorbing organs in mycorhizas, tubercles and other associations. It is obvious that the term saprophyte, or holosaprophyte should be applied only to those species which derive their supply of food from organic products directly without the intervention of the activity of chlorophyll, and unaided by other organisms. In this sense, and it seems to the author to be the only meaning admissible, the holosaprophytes include numerous bacteria and fungi, but so far as present investigations show, only one seed-forming species, *Wullschlaegelia aphylla* : *Cephalanthera Oregana* was erroneously grouped in this class in a previous publication.*

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* An abstract of this discussion of terms was read before the Society for Plant Physiology and Morphology, at New York, Dec. 28, 1898, and was published in American Naturalist, for March, 1899 (10). See also note on same in Science for Feb. 3, 1899, and Botanical Gazette for Feb. and Sept., 1899.

[Issued October 16.]

As a consequence of the above limitations all species furnished with mycorhizas, tubercles, or which enter into direct mechanical or nutritive associations must be classed as symbionts, or if it is desirable to maintain connection with existing literature dealing with these forms it would be permissible to refer to them as symbiotic saprophytes, although such designation must be regarded as tentative, and justified by expediency only (11).

It is a matter of common knowledge that seedlings are holosaprophytic in the stage in which they are wholly dependent upon the reserve material of the seed or fruit, and during the whole period previous to the formation of chlorophyll in general. This period is practically obliterated in those species in which chlorophyll is formed in the seed. On the other hand, the development of this tendency has been twofold. The increase of the capacity for the absorption of organic products has played an important part in the reduction of certain seeds to their present minute form, and again the retention, or extension, of this capacity throughout a greater or less portion of the life of the sporophyte has resulted in varying stages of true saprophytism, complete in one species of the higher plants only. The duration of the holosaprophytic stage shows very wide variations in different species. In certain arums it extends over two years under natural conditions, and may be extended by cultural methods so that the seedling may not form chlorophyll until the third or perhaps even the fourth year of existence, according to experiments now in progress. Only those species which show a marked capacity for the absorption and use of organic products during the greater part of the life of the sporophyte should be classed as hemisaprophytes. The hemisaprophytes would consist chiefly of the carnivorous plants. The greater majority of the species now included in this category are in fact more or less symbiotic by means of mycorhizas, tubercles or other nutritive associations.

This paper describes an extension of the investigations detailed in a previous paper (11), and an effort has been made to determine whether such associations constitute a single physiological type, or whether several types of nutritive adjustment are presented.

Cephalanthera Oregana Reichenb. (13)

A number of living specimens of this plant were examined in the field in Washington and Idaho in 1892, and alcoholic material from this region was obtained in 1899. Besides the notes and material thus obtained, the author has had the opportunity of inspecting herbarium material representing the entire known range of the species, from middle California northward into British Columbia, west of the main continental divide.

The plant consists of an upright subterranean rhizome 5 to 40 cm. in length, from the internodes of which arise the adventitious roots. The internodes are provided with short sheathing scales. The aërial stem is slender, waxy white, 20 to 50 cm. long, and bears short sheathing leaves which are wholly devoid of chlorophyll. The flowers form a dense terminal raceme and perhaps agree with those of other members of the genus in being self-fertilizing (Plate 367, Fig. 1).

The seedling has not been observed. The rhizome is perennial, and the stumps of two or three old aërial stems may be seen adhering to the most recently formed internodes. Although reported from open meadows by some collectors, the author has met it only in the deep humus of coniferous forests. Its deep penetration of the loose substratum, which is generally woven together by the interlacing roots of neighboring trees, makes the collection of the entire plant very difficult and as a consequence the greater number of herbarium specimens are lacking the root system, and on none of these has the writer found the mycorhizal rootlets described below.

The Roots

The roots are wavy cylindrical organs 1.5 to 3 mm. in diameter, and from 5 to 12 cm. in length. One to four arise at each internode of the premorse rhizome, and penetrate the substratum at an angle of forty-five degrees with the horizontal. Mature organs have a yellowish-brown appearance due to the decay of the outer epidermal wall. A many-layered root-cap sheathes the tip for a distance of a millimeter, and shows a fairly normal structure. The epidermal cells are rectangular in surface section, with the radial walls separated to allow a slight outward convexity of the outer surface, which may in some instances assume a papillose form, or may be extended to form typical root-hairs. The latter, and all of the epidermal elements, are rich in protoplasm. The outer walls of the epidermis are covered more or less thickly with crystals of calcium. The sub-epidermal layer is hardly differentiated, and consists of muriform elements rich in protoplasm, and devoid of reserve material. The cortex is composed of ovoid, cylindrical or globose cells, the outer and inner layers of which are composed of elements slightly smaller than those of the middle region. The middle and inner regions are heavily loaded with starch, especially in the older or basal portions, and the cells of the middle region show a tendency to elongation in the radius of the root. Raphide cells are indifferently distributed throughout the cortical tissues, but perhaps more abundantly in the outer layers. Two types of roots which do not differ greatly in outward aspect may be distinguished; a fibrous form, and a fleshy form about twice the diameter of the first. External to the endodermis the two are similar except in the amount of development of the cortex. In the development of the thicker storage organs, the central parenchyma becomes slightly lignified and sclerotized, the xylem bundles increase from six to seven or eight, the pericycle shows two or three layers underneath the endodermis, and this sheath is composed of slightly thickened and pitted elements with no special passage cells. The xylem and the endodermis become lignified (Plate 369, Fig. 2). In the development of the fibrous roots the xylem undergoes such centripetal development, and lateral fusion that it changes from a hexarch to a tetrach. The heavily lignified xylem and the weakly developed central parenchyma form a four-rayed star, with the phloem lodged in the sinuses (Plate 369, Fig. 2). The endodermis is heavily thickened in the regions external to the phloem regions and consists of thin-walled passage cells opposite the xylem. The xylem shows great degeneration in both types of roots, and consists of scalariform vessels and elongated tracheids with transverse pits. The phloem exhibits no great deviation from the normal, and consists of narrow sieve tubes, companion cells and some parenchyma. A layer of elongated elements containing protoplasm and apparently adapted to the conduction of proteids lies immediately outside the phloem proper. The two types of roots show a remarkable resemblance in stelar alterations to those of the two types in *Wullschlaegelia aphylla* as described by Johow (7, p. 427), though not so distinctly separated by external characters.

The older roots often assume a dark-brown color over certain regions 6–10 cm. in length, due to the presence of an ectotropic fungus, which forms a *permanent mycelium* in three or four of the outer layers of the cortex (see page 523). The hyphae are heavy walled and septate. The inner branches form large vesicles which occupy the greater part of the cells of the medio-cortex. The outer branches of the mycelium pass through the short root-hairs into the soil. The region inhabited by the fungus gives rise to a few rootlets which assume the form of lozenge shaped branches with a length not greater than 3 cm. These branches arise endogenously, are brownish to within a few millimeters of the tips and are furnished with a well developed root cap. The mycorhizal fungus advances toward the tips as in the branches of the *Corallorhiza*, and the entire structure shows an arrangement generally similar to the coralloid branches of that plant.

In the half dozen perfect specimens in the hands of the writer the branches arise only from regions inhabited by the fungus and it is conjectured that the presence of this organism may act as a stimulant in setting up such action. The fungus is found in portions of the root-system 50 to 60 cm. below the surface of the soil, and as these organs are very brittle, especially the mycorhizal portions they are generally broken off in collection. The absence of chlorophyll and mycorhizas from numerous specimens sent in by the most careful collectors led the writer to announce that the

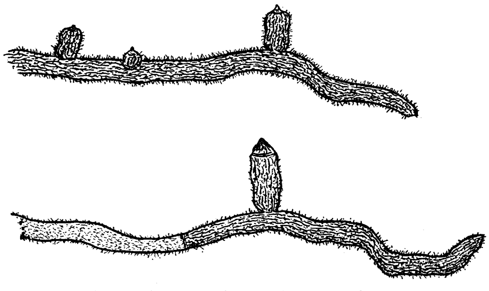

Fig. 1. Mycorhizal portions of roots of *Cephalanthera*.

species was a true saprophyte (11). Recently, however, Mr. O. D. Allen, of Ashford, Washington, by special request, dug up some entire specimens with the result that the mycorhizas were preserved, and are shown in Figure 1.

This species is noted as a parasite in systematic texts, but the author has failed to find any direct evidence of its nutritive connection with any other seed-forming species, either by his observations or from the reports of collectors.

The structural features of the roots agree with those of other mycorhizal forms in the reduction of the absorbing surfaces, the lack of spiral and annular vessels, the formation of tracheids with narrow transverse pits and the radial elongation of the mediocortical region. This last named feature has been supposed to be due to the influence of the fungus in mycorhizal roots, but Groom has shown that it may occur in portions of the root unoccupied by the symbiont. Its presence here, in portions of the root not occupied by the fungus, indicates that it is due to the necessities of absorption and use of organic food perhaps rather than to the stimulation of the presence of a symbiotic organism.

The Rhizome

The rhizome is an upright stem, slightly compressed, with internodes 1 to 2 cm. long, from the terminal internodes of which annually arise one or two flower scapes.

The epidermis is composed of elongated muriform elements with the outer walls slightly convex. No transpiratory openings are to be found. The cortex is ten to fifteen layers in thickness, the cells are ovoid, cylindrical, pitted and separated by spare intercellular spaces. The starch stored here during the resting season is usually exhausted in the formation of the inflorescence. The pericycle is two or three layers in thickness, heavily sclerotized and lignified and is interrupted in places by thin-walled elements. The crowded xylem ring contains a large number of scalariform ducts, and some imperfectly differentiated spiral and annular vessels, all deeply lignified. The phloem consists of cambiform elements with spare protoplasmic content. The interfascicular parenchyma shows collenchymatous thickenings in places, and numbers of elongated conducting cells with unlignified walls are placed near the vessels (Plate 369, Fig. 4).

The rhizome exhibits no marked or general degeneration, unless the condition of the phloem could be interpreted in this manner. The xylem is amply developed for the conduction of the supply of water necessary for the aërial shoot, and the elongated elements near the vessels appear to be suitable for the transmission for proteinaceous compounds, a function served by certain cells immediately internal to the endodermis in the root. The rhizome of *Cephalanthera* differs from similar members in symbiotic saprophytes in not being provided with organs for the excretion of water.

THE INFLORESCENCE

The flowering stem is composed of flattened internodes 2 to 5 cm. long, bearing short sheathing leaves, and a terminal raceme of flowers. The epidermis is composed of flattened cylindrical elements with oblique ends, resembling tracheids in general form, and with the inner and radial walls dotted with numerous perforations. Irregular masses, globules and networks of a yellowish brown substance are abundant in the epidermal and cortical cells of material preserved in alcohol. Ordinary chemical tests are without definite reaction, though Raspail's reagent gave slight indication of proteids in these masses. The epidermis is totally devoid of transpiratory openings.

The cortex is eight to twelve layers in thickness, and consists of elongated cylindrical elements with no intercellular spaces. It appears to serve as a tissue for the storage of water. Internal to the cortex is a sclerenchyma sheath, in the inner margin of which lies a circle of 32 to 40 bundles, and centrally placed is a second ring of six to eight bundles, which are fairly identical with those of the rhizome. Each bundle is enclosed in its own schlerenchyma sheath. The sheath and the xylem are heavily lignified. No marked degeneration is to be seen outside of the lack of chlorophyll, the transpiratory organs, and the intercellular spaces of the cortex. The heavy sclerenchyma sheath is a feature of the aërial stems of the symbiotic saprophytes. The xylem shows a very typical development.

THE LEAVES

The leaves are reduced to sheathing bracts, and are destitute of chlorophyll, as shown by an examination of the alcoholic extract with the micro-spectroscope. The free portion representing the lamina is 1 to 2 cm. in length, and the outer dorsal surface is provided with stomata, the guard cells of which are motile and contain starch (Plate 369, Fig. 5). The possibility that the guard cells may contain a small amount of chlorophyll is not excluded, though it could not be detected by ordinary tests in the specimens examined. The epidermis is composed of muriform cells with the outer walls convex and not cuticularized. The mesophyll consists of two layers of irregularly globoid elements rich in protoplasm, and separated by large air-spaces. A third layer of thin-walled elements, cylindrical in form are to be found lateral to the simple fibrovascular bundles. This tissue is devoid of protoplasm and may serve for the storage of water. The sclerenchyma sheath is incomplete at the point of contact with this supposed storage tissue, thus permitting the ready passage of water between the xylem and the thin-walled cells.

The degeneration of the leaf is seen to consist in the loss of chlorophyll, the lack of differentiation of the mesophyll, and the reduction of the surface. This degeneration has been accompanied by the development of a tissue for the storage of water, and by the retention of the stomata. *Cephalanthera* is to be added to the list of chlorophylless species furnished with motile stomata.

General Considerations

It is evident that the mycorhiza of *Cephalanthera* is to be considered as adventitious or accidental in its occurrence. In a large number of ectotropic forms the fungus gains entrance to the underground organ very early in its development, and then keeps pace with its growth. In this instance, however, it is found only in certain regions, and might be mistaken for a parasite were it not for its characteristic vesicles or organs of interchange. The comparatively small area of the mycorhizal structures suggests that *Cephalanthera* is capable of absorbing largely from the humous products independently.

Calypso bulbosa (L.) Oakes

The author has called attention to the occurrence of adventitious mycorhiza in *Calypso* (11), and cited Lundström's description of these formations, which have been found by him and others in Europe and America. Since that paper was sent to the press a shipment of plants has been received, which had been collected in northwestern United States, and a number of the specimens showed the coralloid mycorhiza. A careful reëxamination has been made of these structures in the light of the generalizations drawn from previous material, and the results are presented below.

The subterranean stem of *Calypso* consists of an ovoid tapering corm 1.5 to 2 cm. in length, comprising two or three internodes. The single ovate, or ovate-cordate leaf is terminal, while the inflorescence arises from the first node below. The plant is reproduced vegetatively by a short offset of such reduced length that the new corm formed from its apical internodes stands upright in contact with the old corm (Plate 367, Fig. 6). The few short roots arising from the base of the corm are mycorhizal as described in the previous paper.

Stem-mycorhiza

The old or spent corms of the preceding season's activity may give rise to offsets from the basal internodes, and these may develop into coralloid structures by the repeated branching due to the development of all the buds, as in *Aplectrum*. The general anatomy of the coralloid formations is too nearly like that of the stem-mycorhizas of *Aplectrum* to warrant description here.

The fungus is seen to be a loose skein of hyphae in the three or four outer layers of the cortex passing outwardly through the thin-walled epidermal cells into the substratum, and do not, so far as observations go, traverse through the nodal trichomes. In this respect *Calypso* differs from other coralloid plants. Occasionally small globular or ovoid structures resembling sporangioles are to be found terminating the branches of the hyphae in the outer cortex. The three or four layers of the medio-cortex are filled with dense masses of interwoven hyphae. The hyphae form irregularly swollen branches upon entering the cells of this region, and one or two of these branches near the nucleus of the cortical cell expands into a vesicle, which in turn gives off a large number of branches nearly filling the cell. The hyphae are unseptate and have definite heavy walls. The form, irregular outlines and in-definite membranes of the hyphae in the medio-cortex led Lundström to believe that the fungus was plasmodial in its earlier stages (9). This appearance is heightened by the adhesion of the cytoplasm of the cortical cell to the hyphae. The inner cortex is free from hyphae, and contains starch in abundance, especially in the apical region. Starch is also present in the outer cortex, but quickly disappears from the cells invaded by the hyphae. The nuclei of the outer cortical cells are nearly normal, but those of the medio-cortex, occupied by the fungus, are hyperchromatic, distorted, and in some instances undergo fragmentation, as in *Peramium* (11). The stele is not differentiated into xylem and phloem, and consists chiefly of cylindrical elements rich in protoplasm, not differing greatly from plerome. The endodermis cannot be made out. The fungus of the coralloid structure and that of the roots are quite similar, but their identity is not established.

A comparison of the specimens which have come under inspection makes it apparent that the tendency to form coralloid mycorhizas may play as important a part in *Calypso* as in *Aplectrum*. The coralloid stems were small in some instances, and attached to the base of corms two years old, while in others their bulk was greater than that of the corm of the previous season to which they were attached. The extreme development was found in one specimen in which the coralloid structure was very large, and the old corm to which it was attached was shrunken to half its original size, but was still sound and normal (Plate 367, Fig. 8). A second offset had sprung from the node nearest the apex of the corm. This offset was about a centimeter in length and bore two roots at the first node while the three upper internodes had begun to swell in the formation of a new corm. The terminal portion bore a rounded cordate leaf and a flower bud. The members of this specimen named in order from the basal end were: stem-mycorhiza, spent corm, offset, roots, developing corm, flower bud and leaf. A comparison of the specimens at hand showed a fairly well established correspondence between variation in the outline of the leaf and the development of the stem-mycorhiza. Such variation might be due to the increased capacity of the plants furnished with coralloid structures for the absorption of humus products.

The variation in the leaf is also accompanied by increase in the length of the offsets, the decrease of the capacity of the storage organs, and the diminution in the number of the roots. The coralloid offsets of *Calypso* have not been seen to give rise to new plants as in *Aplectrum*.

The very great divergence of individuals from the type in leaf and flower characters in this species has been a subject of remark among systematists for many years, and Mr. Heller has recently raised one of the most pronounced forms to specific rank (4). Whether such variations of the shoot are directly correlated with the development of the mycorhizal structures or not can not be definitely stated. The matter may be determined only by the careful examination of specimens in the field throughout the habitat of the species.

CORALLORHIZA ARIZONICA Wats.

(No. 94 of collection of 1898 by MacDougal.)

The general morphology, and occurrence of the fungus of the coralloid branches of *Corallorhiza* have been somewhat fully described by Schacht, Irmisch, Reinke and others, chief attention having been paid to *Corallorhiza Corallorhiza* (L.) Karst (*C. innata*). The results at hand, however, were obtained quite early in the history of investigation upon this subject, and certain details now known to be of great interest were not touched upon in these earlier researches. It was deemed advisable, therefore, to make a reëxamination of the mycorhizal structures of another representative of the genus, with especial attention to the physiological relations of the members of the symbiotic union. Jennings and Hanna (6) have recently published a short paper on *C. innata* in which it is stated that the symbiotic fungus is a "hymenomycete and commonly an argaric." *Clitocybe infundibuliformis* Sch., was found attached to the coralloid formations in one instance, and "*Hysterangium stoloniferum of Tulasne*" in another in a manner indicative of the identity of these species with the symbiotic fungus.

Corallorhiza Arizonica is a native of the upper part of the transition zone and the Canadian zone in the Rocky Mountains. The subterranean portion of the plant consists of a dense mass of coralloid stems which lie as deep as 20 cm. below the surface, and from which extends upwardly a premorse rhizome 10 to 15 cm. in length. The thick aërial stem reaches a height of 15 to 25 cm., is sheathed by membranous leaves, and terminated by a strongly developed racemose inflorescence. The leaves and stem are irregularly colored with blotches of reddish, purplish and brownish tints, and appear to be wholly free from chlorophyll. Specimens with rudimentary aërial stems and others in bloom were collected by the writer on Mormon mountain, and on the San Francisco mountain in Arizona in 1891 and 1898, and preserved in alcohol. The results described below are based upon this material.

The coralloid mycorhiza

The mycorhiza of this plant is a dense mass of club-shaped branches arising from the upright underground rhizome, on which the true roots are to be seen as minute papillae. The germination of the seed has not been observed, and it is impossible to say whether the primary roots are developed or not, or at what stage the symbiotic fungus invades the offsets which constitute the coralloid mass. Reinke figures a young plant, probably of the second year's growth, in which the underground member consists of a coralloid stem only (14). The external anatomy and method of branching need no further description in this species. The rhizome attains a thickness of 4 to 6 mm., with internodes 2 to 4 mm. in length.

The phloem consists of two or three layers of prosenchymatous cells with yellowish thickened walls and slimy contents, most nearly like companion cells. The phloem forms two crescents with the tips nearly touching with the 3 to 5 xylem bundles lying internally, or the phloem may form a complete ring enclosing the xylem. The xylem consists almost entirely of scalariform vessels and one or two tracheids in which the perforations are oval and elongated obliquely. The central parenchyma is made up of short cylindrical cells often richly loaded with starch. The pericycle is present as one or two layers of cambiform cells, and the endodermis forms a sheath of flattened cylindrical elements. External to the stele is a cylinder of cortical tissue 10 to 15 layers in thickness composed of ovoid or globoid elements, with large intercellular spaces, and containing starch during the resting period. The medio-cortex is 15 to 20 layers in thickness and is composed of cells with the radial diameter twice the axial. The outer cortex consists of 3 to 5 layers of very thin-walled elements with the tangential diameter greater than the radial. Both the medio-cortex and the outer cortex are provided with intercellular spaces. The epidermis is composed of flattened cells, irregular in outline, with the lateral and inner walls pitted and the outer wall slightly thickened and brownish in color. It is furnished with a large number of stomata, with the motile guard cells of crescentic form (Plate 368, Figs. 5 and 6) containing starch. These and the large intercellular spaces constitute a very efficient aërating system, and makes the coralloid structure independent of the aërial shoot in transpiration, and at the same time allows free access of atmospheric oxygen. The apices of the rudimentary sheathing leaves are soon converted into a number of blackish shreds and their bases persist as wedge-shaped rudiments with no distinct function. Clumps of large trichomes resembling root-hairs arise from papillae which are infra-axillary to the leaves.

The fungus obtains access to the coralloid offset at quite an early stage of its existence and constantly grows toward the elongating apex forming convolutions of pale gray shining hyphae with numerous septae in the outer cortex. The growth of the hyphae keeps pace with the offset in its slow growth, and they extend forward to the shoulder of the blunt tip of each branch, curving inward at this place toward the embryonic tissue. The hyphae in the outer cortex remain active even in old mycorhiza and may be designated as forming the permanent mycelium. The permanent mycelium is thus in the shape of a sub-epidermal cylinder, and when new branches are formed the sheathing cylinder of the fungus is continued out in it. Branches from the apical portion of the permanent mycelium penetrate the medio-cortex while it is still in an undifferentiated condition, and these branches grow and ramify with the development of the cortical cells until the latter are almost filled with their dense convolutions. For some unknown reason the development of the hyphal branches is greatest immediately internal to the nodal trichomes in the medio-cortex. The hyphal branches are generally cylindrical but occasionally portions become swollen to twice the normal diameter, but no enlargements constituting sporangioles, vesicles or other organs of interchange are to be seen. The cortex of the younger portions of the coralloid structures is filled with starch granules which are slowly corroded by the action of the developing hyphae. Cells in which the hyphae have made many convolutions still contain some starch, but it finally disappears. The hyphae in the medio-cortex a distance from the tip are yellowish and collapsed, but no solid bodies are to be seen as a result of the liberation of their disintegration products in the cortical cells. The protoplasm of the latter is well spent but normal, and the nuclei are normal and active. The permanent mycelium sends out external branches through the trichomes into the soil. The permanent mycelium is, therefore, in the form of a sheathing cylinder with numbers of branches opposite each other extending out into the substratum and into the cortex. It is to be pointed out in this connection that the numerous statements to the effect that the fungus gains access to the interior of the coralloid structure through the trichomes are obviously incorrect. Entrance to the offset in the initial stage of the formation of the coralloid branches is perhaps made in this way, but once inside the branch the permanent mycelium is found which keeps pace with growth of the cortex and sends branches outwardly through the trichomes. The continued and repeated entrance of the fungus through the trichomes is an assumption only, and is based on the necessities of the theory of mycorhizas as fungus traps rather than on the actual facts.

The chemotropic reactions of the fungus as shown by its method of extension are of great interest. The permanent mycelium traverses the coralloid branches in the layers of cortical tissue first differentiated. The tips of the hyphal branches are attracted out through the trichomes, presumably by atmospheric oxygen, or by the humus products, which would increase in concentration from the base of the epidermal cells to the apices of the trichomes. The attraction of the branches into the medio-cortical cells must be due to a carbohydrate, rather than a nuclear product, since it is quite noticeable that all convolutions of the hyphae are made in regions of the cell some distance from the nucleus. The tip of a hypha may pass within its own diameter of the nucleus of the cortical cell with mutual indifference, and only in a small number of instances does the presence of the fungus affect the nucleus. Excretions from the hyphae cause some distortion of a few nuclei, which are also hyperchromatic. The hyphae may be traced around the cell in several circuits. Penetration of the wall and entrance into a neighboring cell is not made until the supply of starch is nearly exhausted, and the solution in the contiguous cell would form a stronger chemotropic stimulus. The portion of the hypha in the wall appears but half the normal diameter of the typical filament, and is nearly colorless even in old formations.

The ascending rhizome

The ascending rhizome consists of four or five napiform segments, each consisting of two or three internodes and representing one season's growth. As each segment is formed it gives rise to an inflorescence from an apical node. Later in the season it forms offsets which reproduce the premorse rhizomes with their coralloid branches. As a consequence of this mode of growth, a dozen plants may be found adherent in a colony, with the coralloid mycorhizas closely crowded in a huge clump.

The epidermis of the rhizome consists of very irregular elements, some of which contain starch at all times. Numerous hyathodes with a central oval or oblong central cell filled with a dense mass of yellowish brown secretion, surrounded by six or seven radially arranged elements are to be seen (Plate 368, Fig. 4). The cortex shows a very copious development, consisting of ovoid or globoid elements with ample air-spaces, and embracing a large number of mucilage cells. This member is therefore furnished with a very efficient mechanism for the extrusion of water: an important provision in an organ devoted to the condensation of carbohydrates of soluble carbohydrates to starch, and this process may continue irrespective of the presence of the aërial shoot. The pericycle forms a dense heavy ring of 7 to 10 layers rich in proteids. The stele in general exhibits a degree of degeneration corresponding with that of the coralloid branches. Its parenchymatous elements as well as the cortex are loaded with starch.

The Inflorescence axis

The epidermis consists of flattened cylindrical cells with oblique ends, are rich in protoplasm, and entirely devoid of stomatal openings. The 10 to 15 layers of cortical tissues are furnished with very large intercellular spaces, which may denote an epidermal transpiration of some importance, though no special adaptation for this purpose could be detected. Crystal cells are scattered throughout the cortex and appear even in the epidermis. The pericycle is composed of several layers of elongated cells with thickened walls and yellowish brown contents. The bundles are scattered in the stele with the xylem and phloem radially arranged. Their degeneration is fairly uniform with that of the rhizome and its branches. The leaves are sheathing, destitute of stomata, and show no differentiation of tissue for food-formation, or transpiration, except that the globular parenchyma shows great intercellular spaces. As noted above they are free from chlorophyll. *C. Arizonica* differs from *C. Corallorhiza* (*C. innata*) in the formation of a bulky premorse rhizome furnished with stomata, the total lack of chlorophyll in the shoot, with absence of stomata, and in the greater degeneration of the stele. The differentiation of the fungal symbiont into a permanent mycelium with short-lived and external and internal branches seems to be a mycorhizal character described here for the first time, though it is present in *C. Corallorhiza* and other species. The hyphae in the coralloid formation of *C. Corallorhiza*, *C. multiflora* and others are applied to the nuclei in the cortical cell while in *C. Arizonica* this is not the case, indicating a difference of chemotropic reaction of the fungus in the two instances.

Relations of the mycorhizal components

The fungus in the coralloid formations of *Corallorhiza* draws its nourishment from two sources; from the humus products of the soil and from the carbohydrates in the cortex. The material thus obtained is used in the construction of extensions of the mycelium and its branches. With the growth and progression of the mycorhiza, the older internal branches of the mycelium which have formed dense masses in the medio-cortex undergo disintegration and the products thus liberated may be drawn in two directions: toward the apex of the mycorhiza and toward the premorse rhizome. On arrival at both places the surplus material is converted into starch. The starch of the rhizome is used in the construction of the reproductive and other branches. The starch in the apex of the coralloid mycorhiza is used in the construction of embryonic tissue and a portion of it remains in the medio-cortex and becomes available to the fungus as a highly advantageous food.

Janse and others have upheld the theory that endotropic mycorhizas are similar in physiological value to leguminous tubercles (11), but the only actual proof adduced in favor of this view is the evidence obtained by Nobbe and Hiltner from experiments with *Podocarpus* (12). That endotropic fungi may coöperate in the fixation of free nitrogen in the roots of plants in which they occupy only a portion of the absorbing system is readily admissible and may be considered as proven. Such an explanation is wholly inadequate to account for the arrangement of the mycorhizal components and transpiratory structures in *Corallorhiza*, however, on purely anatomical grounds. The underground members of this genus are furnished with a complete sub-epidermal sheath of mycelium, which fills every cell of the outer cortex in two or three layers, except a minute area at the tip of the coralloid branch, and usually the 10 to 15 layers of the medio-cortex. It is obviously impossible for the *Corallorhiza* to absorb substances from the soil except through and by the agency of the fungus. The fungus may be capable of accomplishing the fixation of free nitrogen, but that it is not its sole, or its major function in the symbiosis, since all of the food-material of the association must pass through its hyphae; a statement equally true of such forms of ectotropic mycorhiza as those of *Pterospora*, *Monotropa*, etc. The higher plant affords a lodgment for the fungus, from which it sends out absorbent and reproductive branches. Food-material taken in by the fungus is yielded to the higher plant and constitutes its sole supply. To this extent the higher plant is parasitic upon the fungus. But the higher plant accomplishes transformations of chemical energy in the food thus obtained of which the fungus is incapable and yields the elaborated product in an advantageous form in the apex of the mycorhiza, where it serves as a food for the advancing mycelium. The higher plant is, therefore, not a fungus-trap pure and simple, as the association is of great mutual advantage.

The principal conclusions which may be drawn from the facts adduced in the foregoing paper may be briefly stated as follows:

I. The term saprophyte should be applied to those species only which derive their food-supply from organic products, unaided by chlorophyll, and without the intervention of other organisms. The true saprophytes therefore include numerous bacteria, fungi and but one seed-forming species—*Wullschlaegelia aphylla.* The saprophytic capacity of the seedling has been extended to cover periods of varying length in the life of the hemi-saprophytes and with symbiosis has reacted to diminish the tendency to store reserve material in seeds.

II. The degenerations of the true saprophytes are generally parallel to those of mycorhizal forms.

III. *Cephalanthera Oregana* and *Corallorhiza Arizonica* are to be added to the list of chlorophylless plants furnished with stomata.

IV. The offsets of *Calypso* are occasionally converted into coralloid mycorhizas as in *Aplectrum.* The stele of such structures is not differentiated into xylem and phloem. The occurrence of the coralloid mycorhiza is accompanied by variations in the form of the leaves, and of the decrease in the capacity of the storage organs in the specimens examined.

V. *Corallorhiza Arizonica* exhibits greater development of the symbiotic adaptation than *C. Corallorhiza.* The stele is quite primitive throughout, chlorophyll is lacking, and stomata are present on the coralloid branches only. The epidermis of the pre morse rhizome is furnished with hyathodes. The aërial shoot is furnished with large intercellular spaces, but may carry on epidermal transpiration only. The fungus in the coralloid structures consists of a permanent mycelium, with external and internal branches; the former are organs of absorption and reproduction, the latter are organs of interchange between the members of the symbiosis. The higher plant affords lodgment for the fungus and carries on chemical transformations the products of which are available to the fungus. The latter absorbs and yields to the higher plant in a more or less complex form the products of the humous soil.

VI. All endotropic mycorhizas do not conform to a single physiological type. The theory of Janse that endotropic fungi are negatively chemotropic to oxygen, and bear the same relation to the higher plant as the organism of the leguminous tubercle, is not capable of general application. Such relation has been proven between *Podocarpus* and the peronosporous fungus of its mycorhiza only.

VII. Two types of endotropic mycorhizas may be distinguished; one adapted for nitrogen fixation, and a second for the absorption and modification—perhaps oxidation—of the soil products before liberation in the tissues of the higher plant. The extension of information will doubtless result in the further division of the second type.

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Explanation of Plates

Plate 367. (1) Entire specimen of *Cephalanthera Oregana* Reichenb: *A*, base of aërial stem. (2) Premorse rhizome of *Corallorhiza Arizonica: A*, terminal bud; *B*, *B*, *B*, *B*, scars of preceding buds; *C* and *D*, offsets from which arise coralloid branches. (3, 4 and 5) Coralloid branches of *Corallorhiza Arizonica*. (6) Typical specimen of *Calypso borealis*: *A*, young corm; *B*, corm of previous season's formation. (7) Aberrant specimen of *Calypso*: *A*, old corm with coralloid branch. (8) Widely aberrant form of *Calypso*: *A*, young corm; *B*, old corm with large coralloid branch, *C*.

Plate 368. *Corallorhiza Arizonica* Wats. (1) Longitudinal section of tip of old coralloid mycorhiza: *a*, *a*, epidermis; *b*, *b*, medio-cortex, containing disintegrating branches of fungus; *c*, stele; *m*, *m*, permanent mycelium; *d*, *d*, branches; *e*, leaf. (2) Portion of transverse section of mycorhiza: *e*, epidermis; *c*, permanent mycelium; *m*, internal branches in medio-cortex; *p*, inner cortex; *b*, phloem; *o*, xylem. (3) Cells from cortex: *a*, *a*, hyphae of permanent mycelium; *c*, masses of hyphae in medio-cortical cells; *n*, *n*, *n*, nuclei of cortical cells. (4) Hyathode from premorse rhizome. (5) Stomata from coralloid branch. (6) Section of epidermis of coralloid branch showing structure of stoma: *e*, *e*, epidermal cells; *a*, guard cells; *n*, nucleus. (7) Transverse section of aërial stem: *e*, epidermis; *c*, cortex; *d*, sclerenchyma sheath; *x*, *x*, fibrovascular bundles.

Plate 369. *Cephalanthera Oregana* Reichenb. (1) Transverse section of stele of storage root: *p*, medulla; *x*, *x*, xylem; *c*, endodermis. (2) Transverse section of stele of fibrous root: *v*, passage cells of endodermis; *e*, thickened cells of endodermis outside of phloem; *s*, phloem; *z*, xylem; *c*, medulla. (3) Longitudinal section in stele; *o*, *s*, vessels; *m*, medulla; *n*, endodermis. (4) Portion of transverse section of rhizome: *e*, epidermis; *h*, cortex; *f*, sclerenchyma sheath; *g*, fibrovascular bundles with heavy sheath; *r*, parenchyma. (5) Stoma from leaf.